Mediterranean Marine Science

Vol 25, No 3 (2024)

Mediterranean Marine Science

First report of Phyllorhiza punctata von Lendenfeld, 1884 (Cnidaria: Scyphozoa, Mastigiidae) in the Balearic Islands (Western Mediterranean Sea)

RAUL GONZÁLEZ, GAETANO CATANESE, MAITE VÁZQUEZ-LUIS

doi: 10.12681/mms.38075

Short Communication

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
www.hcmr.gr
DOI: https://doi.org/10.12681/mms.38075

First report of *Phyllorhiza punctata* von Lendenfeld, 1884 (Cnidaria: Scyphozoa, Mastigiidae) in the Balearic Islands (Western Mediterranean Sea)

Raúl GONZÁLEZ [1], Gaetano CATANESE [2,3] and Maite VÁZQUEZ-LUIS[1]

[1] Instituto Español de Oceanografía (IEO-CSIC). Centro Oceanográfico de Baleares, 07015, Palma de Mallorca, Spain
[2] IRFAP-LIMIA, Institut de Recerca i Formació Agroalimentària i Pesquera de les Illes Balears,
Laboratori d'Investigacions Marines i Aqüicultura, Govern de les Illes Balears, 07157 Port d'Andratx, Spain
[3] INAGEA-UIB, Agro-Environmental and Water Economics Research Institute, University of the Balearic Islands, 07122 Palma, Spain

Corresponding author: Raúl GONZÁLEZ; raul.gonzalez@ieo.csic.es

Contributing Editor: Stelios SOMARAKIS

Received: 10 June 2024; Accepted: 25 September 2024; Published online: 04 November 2024

Abstract

The white-spotted jellyfish *Phyllorhiza punctata* was first recorded in 1965 in the Mediterranean Sea. Here, we describe the first record of *P. punctata* in the Balearic Islands, specifically at the coastal lagoon Estany des Ponts, Alcudia, Mallorca. An adult individual and several juveniles were found in the summer months (July and August) of 2023, suggesting a preference for an increase in physicochemical variables, such as salinity and temperature. Molecular analysis performed on an adult individual allowed its genetic characterization. The small size and the low number of individuals found at this site suggest a recent invasion of this area of the Mediterranean Sea. Possible pathways for *P. punctata's* arrival in this area have been suggested, but further monitoring is required to evaluate the evolution of *P. punctata* communities in this region in the subsequent years and to establish conservation strategies in the upcoming years.

Keywords: *Phyllorhiza punctata*; jellyfish; biological invasions; invasive species; non-indigenous species (NIS); alien species.

Introduction

The white spotted jellyfish *Phyllorhiza punctata* von Lendenfeld, 1884 (Cnidaria: Scyphozoa: Rhizostomeae) belongs to the Mastigiidae family within Scyphozoans. It was first recorded in 1965 in the Mediterranean Sea (Galil *et al*., 1990). In addition to the morphological description provided by Ocaña-Luna *et al*., (2010) this taxon is characterized by white exumbrellar dots. Below the umbrella, there are eight oral arms (whose color may vary due to the presence of zooxanthellae) (Morandini *et al*., 2006) culminating in large brown bundles of stinging cells that are a relatively low stinging hazard for humans (Graham *et al*., 2003). Indirect impacts associated with *P. punctata*'s presence in new areas and existing trophic chains include limiting mesozooplankton abundance (García & López, 1989; García, 1990; Gueroun *et al*., 2015) and interfering with the commercial shrimp industry by clogging the nets (Ocaña-Luna *et al*., 2010).

P. punctata is considered an invasive exotic species, native of the Indo-Pacific region (Heeger *et al*., 1992; Graham *et al*., 2003). It has been reported outside its natural distribution several times (Ocaña-Luna *et al*., 2010) forming stable populations. For instance, the transportation of *P. punctata* polyps on ship hulls seemed to be the key vector for introducing this species into Californian waters in 1981 (Larson & Arneson, 1990). Something similar occurred in the eastern Pacific and the western Atlantic where ships played a key role in spreading *P. punctata* through ballast water or fouling (Rosales-Catalán *et al*., 2021).

Reportedly, *P. punctata* has reached the Mediterranean Sea through the Suez Canal (Lessepsian migrant). Although *P. punctata* populations remain scarce in the Mediterranean Sea, the fast spread of *P. punctata* has been remarkable in the entire basin (Kaminas *et al*., 2022; Fernández-Alías *et al*., 2024) since the early report of its presence in Israel (Galil *et al*., 1990).

This is the first report of the presence of *P. punctata* in the Balearic Islands (western Mediterranean Sea) attempting to improve the knowledge of this alien jellyfish in the Mediterranean Sea. Furthermore, we contribute to their genetic characterization of this species by integrating a multidisciplinary approach.

Materials and Methods

Study area and data collection

The new findings of this study correspond to the coastal lagoon "Estany des Ponts" (Fig. 1) within the Albufera de Mallorca in the Balearic Islands. This area was a part of the wetlands of Alcudia Bay until its alteration in the nineteenth century. The area is included in the "*Inventario Balear de Zonas Húmedas*". This lagoon is currently under pressure due to anthropogenic impact (WWF, 2018).

Since May 2023, monthly surveys have been conducted to search for *P. punctata* individuals in the area (either by spotting them from the shore or snorkelling) while measuring physicochemical variables (salinity and temperature). During these surveys, the specimens spotted were photographed for further processing with ImageJ v1.54 software to determine the bell size. Due to logistic issues, the genetic analysis was only carried out with only one individual (Table 1). The specimen was collected by hand, trying to avoid damaging the body when removing it from the water (Fig. 2A). After collection, a piece of the organism was placed into 96% Ethanol (CH_3CH_2OH) for molecular analysis; the rest of the individual was preserved in 4% Formaldehyde (CH_2O) for morphological inspection. Samples were transported to the laboratory where they were stored at room temperature and -20 ºC for further processing.

Molecular analyses

The DNA extraction was carried out on samples of tissue using the commercial Macherey-Nagel DNA Tissue extraction kit (Düren, Germany), following the manufacturer's instructions. The quality and concentration of the DNA were measured using the Nanodrop ND1000 (Thermo Scientific, Waltham, MA, USA).

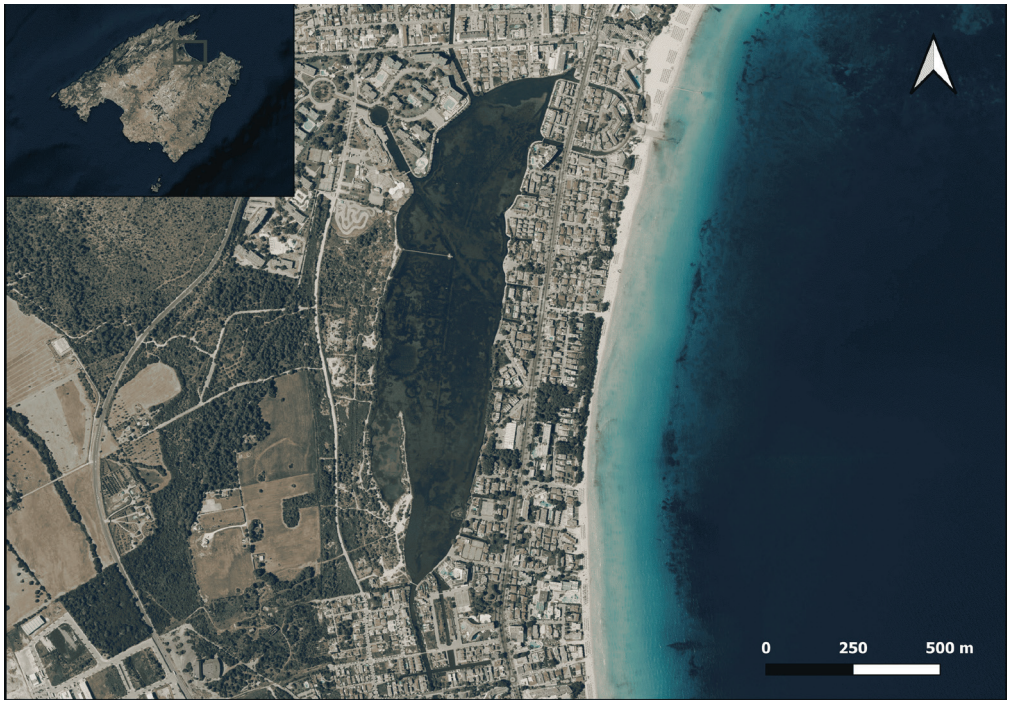

Fig. 1: Study area and finding location of *Phyllorhiza punctata.*

Table 1. New findings of *P. punctata* in the Balearic Islands.

Sighting date	Population status (*P. punctata*)	Salinity range (PSU)	Temperature range (ºC)	Umbrella diameter (mm)	GeneBank Accession number
08-05-2023	-	26.1-28.6	23.4-25.6	-	-
08-06-2023	-	28.8-31.4	23.9-28.8	-	-
12-07-2023	15-20 specimens	32.7-36.5	28.5-33.0	20–25	-
12-08-2023	Single specimen	35.5-37.9	24.7-28.6	77	LC814843
13-09-2023	-	33.9-36.1	26.3-28.8	-	-
11-10-2023	-	32.6-35.6	23.0-26.1	-	-
17-01-2024	-	32.7-36.0	13.7-15.1	-	-
08-03-2024	-	27.5-28.8	15.4-17.0	-	-

Fig. 2: Data collection during surveys within the study area. A-Sample collection of *Phyllorhiza punctata* adult individual. B-EXample of anthropogenic waste found during the surveys.

For species identification, a fragment of approximately 650 bp of the mitochondrial cytochrome c oxidase subunit I gene (barcoding) was amplified by PCR and sequenced using the universal primers HCO2198-LCO1490 (Folmer *et al*., 1994). PCR reactions were performed in a total volume of 20 µL containing 10 µL KAPA Taq Ready Mix PCR kit (Sigma-Aldrich, Burlington, MA, USA), 0.4 µL of each primer (stock 20 µM), and 1 µL of DNA (20 up to 80 ng/µL) and water to make up the final volume. The temperature protocol consisted of an initial denaturation step at 94 °C for 2 min, and 40 cycles of 94 °C for 30 s, 50 °C for 20 s, and 72 °C for 1 min. PCR products were separated on 1.5% agarose in TAE 1x buffer gels, including a HighRanger 1 kb DNA ladder size standard, stained with GelRed (Biotium, Fremont, CA, USA) (Norgen, Thorold, Canada), and visualized on a UV transilluminator.

Amplicons were extracted from the gels and purified using a mi-PCR purification Kit (Metabion International, Planegg, Germany) following the manufacturer's instructions. All PCR fragments were bi-directionally sequenced using the 3130xl DNA automated sequencer (Applied Biosystems, Carlsbad, CA, USA) at Secugen S.L. service (Madrid, Spain). Finally, the sequences were edited and aligned using BioEdit v7.2.5 software (Hall, 1999) to perform a comparative analysis with other known sequences in GenBank, using the BLAST application.

Results and Discussion

The consensus sequences were deposited in GenBank under accession number LC814843. BLAST results showed similarity with other sequences obtained from the Balearic waters, particularly with sequences of *Phyllorhiza spp*. (99.2% identity; Access. numb. MT904380) and *Phyllorhiza punctata* (99.05% identity; Access. numb. GQ120101).

In the approximately 600 bp fragment analyzed, we detected 10 nucleotide differences compared to the *P. punctata* sequence present in GenBank. Furthermore, we observed double peaks at eight nucleotide positions, specifically indicated as Y (C or T) at positions 144, 303, and 447, or R (A or G) at positions 273, 333, 336, 447, and 483 of the previously reported sequence. These findings suggest the presence of potential heteroplasmy within the species as seen in other invertebrate species (Rodríguez-Pena *et al*., 2020). However, more samples should be analyzed to confirm this hypothesis.

Since May 2023, eight surveys have been carried out in the study area; *P. punctata* individuals were spotted only in two of these surveys (Table 1). The range of bell sizes varied from 20 to 77 mm. No specimens over 77 mm have been found in this location. This is in contrast to reports from other regions, where umbrella diameters exceed 100 mm (Graham *et al*., 2003; Abed-Navandi & Kikinger, 2007; Galil *et al*., 2009; Enrique-Navarro & Prieto, 2020).

The bell size of the young individuals spotted in July (20-25mm) was similar to that of juvenile individuals found in Rio San Pedro tidal creek (RSP) (Cadiz, Spain); while the size of the single individual spotted in August was within the size of the adults found in RSP (Enrique-Navarro & Prieto, 2020). The small size and the low number of individuals observed may indicate a recent invasion of this area of the Mediterranean Sea and the absence of an established population, unlike other regions in the Mediterranean Sea where a high number of sexually mature specimens have been reported (Galil *et al*., 2009; Gueroun *et al*., 2015; Kaminas *et al*., 2022).

The juvenile specimens photographed presented a blue pigmentation of the bell (Fig. 3A-B) and terminal clubs, similar to the juvenile individuals found at RSP (Enrique-Navarro & Prieto, 2020). Neither this pigmentation nor the light brown color background of the bell was found in the adult individual (Fig. 3C-D), although these are characteristic of the zooxanthellae symbiosis found in other adult individuals (Ocaña-Luna *et al*., 2010; Gueroun *et al*., 2015; Stamouli *et al*., 2018). This morphological variability has been previously reported concerning the presence or absence of zooxanthellae (Bolton & Graham, 2004; Madkour *et al*., 2021), whose absence leads *P. punctata* to feed on zooplankton (Graham *et al*., 2003).

In the surveys conducted in the area, *P. punctata* individuals were only found in the summer months (Table 1), which may suggest a preference for the physicochemical properties of the water present during these months. A similar phenomenon has been reported in Mexico, where this species shows a seasonal pattern: their numbers rise in the spring and summer months and decline in the rainy and cold seasons, especially when the salinity is below

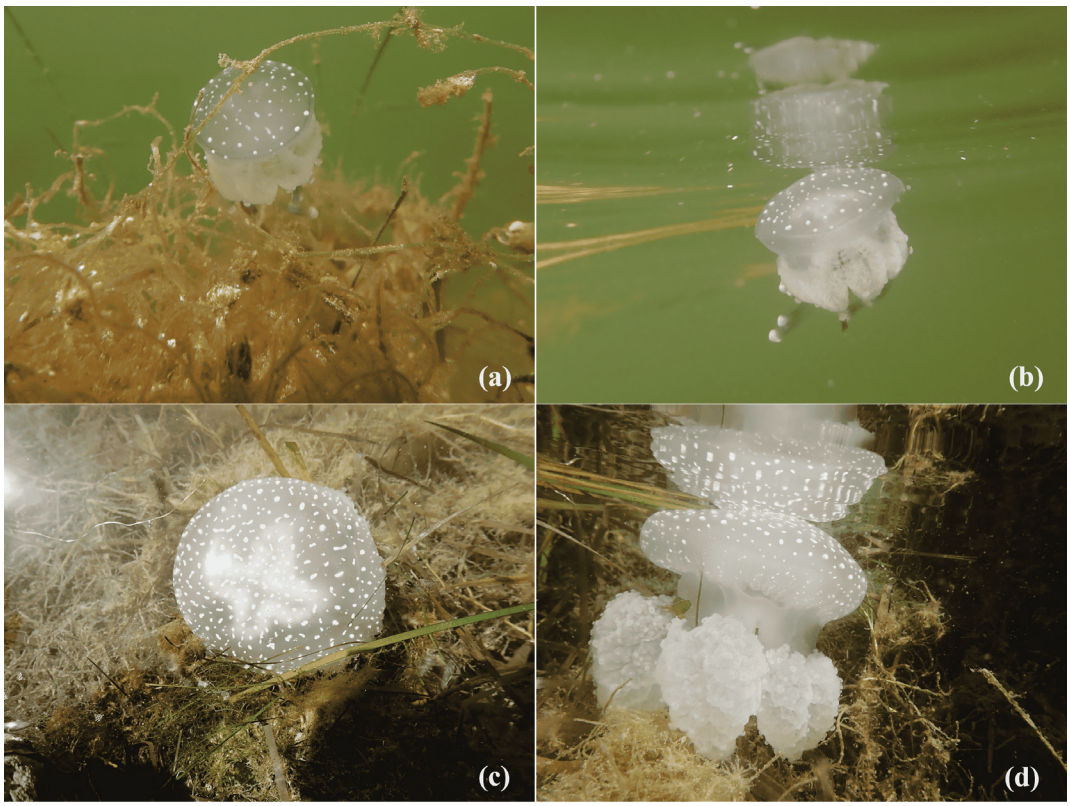

Fig. 3: *Phyllorhiza punctata* individuals found during the surveys in summer 2023. A-B, young individuals found in July 2023; C-D adult individual found in August 2023 on which the genetic analysis was performed.

10 psu (Ocaña-Luna *et al*., 2010). Although this species tolerates a wide range of temperature and salinity conditions, the absence of individuals during the rainy and cold seasons in the Estany des Ponts may signal non-optimal conditions for the proliferation of *P. punctata* in surface waters, like in the Swan-Canning estuary in western Australia (Rippingale & Kelly, 1995). Unlike other regions, the shallow depth of Estany des Ponts limits *P. punctata* from seeking deeper shelter from alluvial rain and storms during the rainy and cold seasons (Martínez *et al*., 1989). Nonetheless, *P. punctata* adults and ephyra individuals have been detected in shallow coastal lagoons such as the Mar Menor in Murcia (Fernández-Alías *et al*., 2024). Thus, further monitoring is required to evaluate the evolution of *P. punctata*'s population in our study area in subsequent years, as the increase in temperature and salinity (especially in the summer months) may cause a sudden proliferation of this species.

Since its first detection in 1965 in Israel (Galil *et al*., 1990), *P. punctata* seems to have spread and established in several regions of the eastern Mediterranean Sea (Galil *et al*., 2009). In the western Mediterranean Sea, *P. punctata* was spotted for the first time in Italy in 2009 (Boero *et al*., 2009). Three years later, it was detected for the first time on the coasts of Tunisia and Spain with more sightings in subsequent years (Barrado *et al*., 2014; Gueroun *et al*., 2015). While Madkour *et al.* (2021) anticipated the possible spread of this species into the western Mediterranean Sea, some possible mechanisms for P. punctata's spread in the Mediterranean Sea have been proposed (Fernández-Alías *et al.*, 2024). This tropical jellyfish is among the top three most invasive species of the 45 jellyfish species assessed by Killi *et al*., (2020). Some of the reported impacts of *P. punctata* include decreased abundance of zooplankton and the clogging of nets in the shrimp industry (García & López, 1989; García, 1990; Ocaña-Luna *et al*., 2010; Gueroun *et al*., 2015).

P. punctata's tolerance to changes in environmental parameters and its abundance in coastal zones such as estuaries, lagoons, and bays (Graham *et al*., 2003) makes the monitoring of these environments necessary for the evaluation and management of this tropical species. As Fernández-Alías *et al*., (2024) pointed out, the role of semi-enclosed habitats as stepping-stones in the *P. punctata* invasion of the Mediterranean Sea is crucial to understanding their colonization patterns (Carlton, 1996).

Our findings constitute the first record of this non-native species in the Balearic Islands and confirm the spread of the species throughout the Mediterranean Sea. Therefore, the monitoring of this species as well as studies on its population dynamics are necessary to establish and evaluate effective management and conservation strategies for the upcoming years.

Acknowledgements

We would like to acknowledge the Tragsatec 09 team and the environmental agents of the Ministry for Ecological Transition and Demographic Challenge (MITECO) for their assistance during field surveys. We would also like to thank the staff of "Albufera de Mallorca" Natural Park for their willingness to provide information about the "Estany des Ponts".

Author contributions: Conception, design, and collection of field data were conducted by RG and MVL. Analysis and interpretation of genetic data were performed by GC, while RG and MVL handled the environmental data. All authors contributed to the drafting of the initial manuscript, its subsequent revision, and the review and approval of the final version. **Funding:** This work was supported by the European Union's LIFE program through the project LIFE PINNARCA (NAT/ES/001265). **Data availability:** The data that support the findings of this study are available from the corresponding author upon reasonable request. **Conflicts of interest**: The authors declare no conflicts of interest.

References

Abed-Navandi, D., Kikinger, R., 2007. First record of the tropical scyphomedusa Phyllorhiza punctata von Lendenfeld, 1884 (Cnidaria: Rhizostomeae) in the central Mediterranean Sea. *Aquatic Invasions,* 2 (4), 391-394.

Barrado, C., Fuentes, J.A., Salamí, E., Royo, P., Olariaga, A.D. *et al*., 2014. Jellyfish monitoring on coastlines using remote piloted aircraft. 17 (1), 012195. In: IOP Conf. Series: Earth and Environmental Science. IOP Publishing.

Boero, F., Putti, M., Trainito, E., Prontera, E., Piraino. S. *et al*., 2009. First records of Mnemiopsis leidyi (Ctenophora) from the Ligurian, Tyrrhenian and Ionian Seas (Western Mediterranean Sea) and first record of Phyllorhiza punctata (Cnidaria) from the Western Mediterranean Sea. *Aquatic Invasions*, 4 (4), 675-680.

Bolton, T.F., Graham, W.M., 2004. Morphological variation among populations of an invasive jellyfish. *Marine Ecology Progress Series*, 278, 125-139.

Carlton, J.T., 1996. Pattern, process, and prediction in marine invasion ecology. *Biological conservation,* 78 (1-2), 97-106.

Enrique-Navarro, A., Prieto, L., 2020. First record of Phyllorhiza punctata (Cnidaria: Rhizostomae: Mastigiidae) in the Northeast Atlantic Ocean. *Cahiers de Biologie Marine*, 61 (3), 349-353.

Fernández-Alías, A., Quispe-Becerra, J.I., Conde-Caño, M.R., Marcos, C., Pérez-Ruzafa, A., 2024. Mediterranean Sea biogeography, colonization, expansion, phenology, and life cycle of the invasive jellyfish Phyllorhiza punctata von Lendenfeld, 1884. *Estuarine, Coastal and Shelf Science*, 299, 108699.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3 (5), 294-299.

Galil, B.S., Spanier, E., Ferguson, W.W., 1990. The scyphomedusae of the Mediterranean Sea coast of Israel, including two Lessepsian migrants new to the Mediterranean Sea. *Zoologische Mededelingen (Leiden)*, 64 (7), 95-105.

Galil, B.S., Shoval, L., Goren, M., 2009. Phyllorhiza punctata von Lendenfeld, 1884 (Scyphozoa: Rhizostomeae: Mastigiidae) reappeared off the Mediterranean Sea coast of Israel. *Aquatic Invasions,* 4 (3), 481-483.

García, J.R., 1990. Population dynamics and production of Phyllorhiza punctata (Cnidaria: Scyphozoa) in Laguna Joyuda, Puerto Rico. *Marine Ecology Progress Series,* 64, 243-251.

García, J.R., López, J.M., 1989. Seasonal patterns of phytoplankton productivity, zooplankton abundance and hydrological conditions in Laguna Joyuda, Puerto Rico. 53 (2-3), p. 625-631 In: *Scientia Marina*, *Barcelona*, 1989. Consejo Superior de Investigaciones Científicas (CSIC).

Graham, W.M., Martin, D.L., Felder, D.L., Asper, V.L., Perry, H.M., 2003. Ecological and economic implications of a tropical jellyfish invader in the Gulf of Mexico. *Biological Invasions* 5, 53-69.

Gueroun, S.K.M., Kéfi-Daly Yahia, O., Deidun, A., Fuentes, V., Piraino, S., *et al*., 2015. First record and potential trophic impact of Phyllorhiza punctata (Cnidaria: Scyphozoa) along the north Tunisian coast (South Western Mediterranean Sea). *Italian Journal of Zoology*, 82 (1), 95-100.

Hall, T.A., 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series,* 41, 95-98.

Heeger, T., Piatkowski, U., Möller, H., 1992. Predation on jellyfish by the cephalopod *Argonauta argo*. *Marine Ecology Progress Series,* 88, 293-296.

Kaminas, A., Shokouros-Oskarsson, M., Minasidis, V., Langeneck, J., Kleitou, P. *et al*., 2022. Filling gaps via citizen science: Phyllorhiza punctata von Lendenfeld, 1884 (Cnidaria: Scyphozoa: Mastigiidae) in Cyprus (eastern Mediterranean Sea). *BioInvasions Records,* 11 (3), 667-675.

Killi, N., Tarkan, A.S., Kozic, S., Copp, G.H., Davison, P.I. *et al*., 2020. Risk screening of the potential invasiveness of non-native jellyfishes in the Mediterranean Sea. *Marine Pollution Bulletin*, 150, 110728.

Larson, R.J., Arneson, A.C., 1990. Two medusae new to the Coast of California: Carybdea marsupialis (Linnaeus, 1758), a cubomedusa and Phyllorhiza punctata von Lendenfeld, 1884, a rhizostome scyphomedusa. *Bull., Southern California Academy of Sciences,* 89 (3), 130-136.

Madkour, F., Elnagar, N., Killi, N., 2021. Re-occurrence of the invasive jellyfish Phyllorhiza punctata von Lendenfeld, 1884 in the coast of Egypt and spreading pattern in the Mediterranean Sea. *Biharean Biologist*, 15 (2), 87-90.

Martínez, A., Díez, A., Forteza, V., Ramón, G., Sastre, A. *et al*., 1989. Característiques morfomètriques dels principals estanys de l'Albufera de Mallorca. *Treballs de Geografia*, 19-32.

Morandini, A.C., Soares, M.O., Matthews-Cascon, H., Marques, A.G., 2006. A survey of the Scyphozoa and Cubozoa (Cnidaria, Medusozoa) from the Ceará coast (NE Brazil). *Biota Neotropica,* 6 (2), 1-8.

Ocaña-Luna, A., Sánchez-Ramírez, M., Aguilar-Durán, R., 2010. First record of Phyllorhiza punctata von Lendenfeld, 1884 (Cnidaria: Scyphozoa, Mastigiidae) in Mexico. *Aquatic Invasions*, 5 (1), S79-S84.

Rippingale, R.J., Kelly, S.J., 1995. Reproduction and survival of Phyllorhiza punctata (Cnidaria: Rhizostomeae) in a seasonally fluctuating salinity regime in Western Australia. *Marine and Freshwater Research,* 46 (8), 1145-1151.

Rodríguez-Pena, E., Verísimo, P., Fernández, L., González-Tizón, A., Bárcena, C. *et al*., 2020. High incidence of heteroplasmy in the mtDNA of a natural population of the spider

crab Maja brachydactyla. *PloS one*, 15 (3), e0230243.
Rosales-Catalán, L., Estrada-González, M.C., Rivera-Pérez, C., Sanchez, M.A.R., Gamero-Mora, E. *et al*., 2021. Genetic and morphological evidence of the presence of Phyllorhiza punctata in the southwestern Gulf of California (NE Pacific Ocean). *Aquatic Invasions,* 16 (4), 637-652.
Stamouli, C., Akel, E.H.K., Azzurro, E., Bakiu, R., Bas, A.A. *et al*., 2018. New Mediterranean Sea Biodiversity Records (December 2017). *Mediterranean Sea Marine Science,* 18 (3), 534-556.
WWF España, 2018. Informe del Inventario: ES532MAL026 - Estany des Ponts. EsIsWet - Base de datos de los humedales insulares españoles.https://www.humedalesdebaleares.es/general/report.php?code=MAL026&lang=es_ES (Accessed in 04.03.2024) Updated: 05.2023.